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(54) Transdermal treatment with mast cell degranulating agents for drug-induced hypersensitivity.

(57) Methods and compositions for inhibiting or preventing the skin irritating or sensitizing effects of a skin irritating or sensitizing component of a dermal or transdermal drug delivery system are disclosed. The composition comprises a mast cell degranulating agent which is capable of inducing a state of immunological tolerance to the skin sensitizing agent by delivery prior to, or at the onset of transdermal drug delivery. Such an agent, preferably *cis*-urocanic acid or an analogue or metabolite thereof, can be administered before, during or after each transdermal drug delivery to achieve immune tolerance countersensitization. Alternatively, the agent can be used to induce countersensitization. The agent is preferably capable of permeating the epidermis and is administered transdermally. Novel methods and compositions comprising *cis*-urocanic acid or an analogue or metabolite thereof to obtain anti-inflammatory effects are also disclosed.

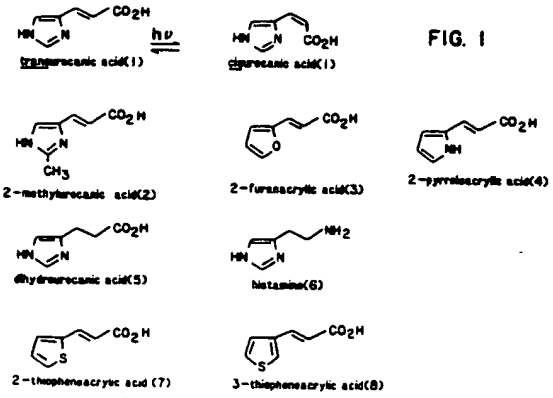

FIG. 1

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(1987)).

Nonionic surfactants, in particular polyoxyethylenes, used in TDS or considered useful as solubilizers, plasticizers or penetration promoters, caused irritation (Mezei, M. *et al., J. Pharm. Sci. 59*:129 (1970)). Methylmethacrylate, a starting material for polyacrylate-based TDS, showed dermatotoxic reactions upon dermal absorption (Kanerva, L. *et al., Arch. Toxicol. Suppl. 9*:456 (1986)).

In summary, it is a likely risk that many small molecules, once incorporated into a TDS, encounter ideal conditions for entering the viable epidermis and triggering an allergic response. This risk is enhanced due to a most basic property of almost all TDS: occlusivity.

2.2. TREATMENT OF DRUG-INDUCED SKIN SENSITIZATION

In reviewing the TDS field, Bodde, H.E. *et al., Crit. Rev. Ther. Drug Carrier Syst. 6*:87-115 (1989), listed a number of approaches for avoiding undesired drug side effects in the skin. Included in this list were the avoidance of irritating or sensitizing components, appropriate testing of allergenic drugs, avoidance of friction and pressure, and minimization of occlusion. The only pharmacotherapeutic approach, mentioned in passing, was the suppression-of skin reactions by delivering antihistamines or cromolyn sodium by the TDS.

Others have addressed the problem of treating or preventing skin inflammation produced by the transdermal delivery of a skin-irritating and/or a skin-sensitising drug. For example, Ross *et al.*, U.S. Patent #4,897,260 (30 Jan. 90), EP 0282156 and WO 88/09175 disclosed that the addition of glucocorticoid carboxylic acid esters, such as triamcinolone acetone 21-oic acid methyl ester, to percutaneous drug delivery devices suppressed the induction of cutaneous DH by a component in a percutaneous drug delivery system. Patel, D.C. *et al.*, U.S. Patent 4,855,294, disclosed that glycerin reduced mild skin irritation caused by drug permeation enhancers, such as alcohols, diols, oleic acid, and glycerol dioleate.

Cormier *et al.*, U.S. Patent 4,885,154 (5 Dec. 89) disclosed reduction in skin or mucosal sensitization or irritation by the transdermal co-administration, with a sensitizing or irritating drug, of a "metabolic modulator." Such modulators included tranylcypromine and phenyl alcohols, for example, as 2-phenyl-1 ethanol, 3-phenyl-1-propanol, 4-phenyl-1-butanol and cinnamyl alcohol. The basis of the choice of modulator was its ability to inhibit the enzyme that metabolizes the drug, which presumably prevented the generation of an irritating or allergenic drug metabolite. The combined administration of an immunogenic drug and such modulators also inhibited sensitization by the drug..

Amkraut *et al.*, U.S. Patents # 5,000,956 (19 Mar. 91) and 5,077,054 (31 Dec. 91) disclose the use of a corticosteroid, co-extensively co-administered with a sensitizing agent, to prevent contact allergy induced by the transdermal delivery of a sensitizing drug. Preferred corticosteroids were hydrocortisone and its esters. Amkraut, U.S. Patents 5,049,387 (17 Sept 91) and 5,118,509 (2 Jun 92), disclosed a method for inducing immune tolerance to a sensitizing drug by continuously and co-extensively co-administering to a skin or mucosal site the drug and a corticosteroid, preferably hydrocortisone or an ester thereof. After such a regimen, when the sensitizing drug is administered, the subject is non-reactive. Also disclosed are transdermal delivery systems for the tolerance producing combinations.

Ledger *et al.*, U.S. Patent 5,120,545 (9 Jun 92) discloses a method for reducing or preventing skin sensitization by inhibiting the immunological processing of a transdermally sensitizing drug as an antigen. The method comprises co-administration to the skin or mucosa of the sensitizing drug and an agent which inhibits antigen processing. The inhibition of antigen processing could occur either in the induction phase or the elicitation phase of skin sensitization. Effective inhibiting agents were said to be those which raised the intracellular pH of low pH organelles, particularly the intralysosomal pH of antigen presenting cells in the skin. Examples of such agents are ionophores which interfere with ionic pumps and thereby allow intralysosomal pH to rise. Alternatively, inhibitors could be weak bases which accumulate and raise the pH in lysosomes. Example of such compounds are amphiphilic cations including amphiphilic amines such as ammonia and its salts (in particular ammonium chloride), low molecular weight amines and their salts, and amino alcohols, such as ethanolamine, diethanolamine, triethanolamine and tromethamine, and their salts .

Cormier *et al.*, U.S. Patent 5, 130,139 (14 Jul 92), contains a disclosure similar to Ledger *et al., supra*, additionally disclosing the co-administration of a lysosomal uptake inhibiting agent to block sensitization with a weak base irritating drug. Preferred inhibiting agents were monensin and amphiphilic amines, including ammonium chloride. Ammonium chloride and monensin reduced the irritation induced by propranolol or chloroquine without inhibiting drug permeation into the skin.

South African Patent Application ZA 9006583 (to Beth Israel Hospital) discloses the use of the calcium channel blocker nifedipine as a countersensitizer and skin inflammation inhibitor. Nifedipine was shown to significantly reduce the inflammation caused by epicutaneously challenging mice previously sensitized with

M. L. *et al.. J. Immunol. 137*:443 (1986); Yoskikawa, T. *et al., J. Invest. Dermatol. 95*:530 (1990)). These UVB effects are thought to be mediated, in part, the UVB-induced isomerization of *trans*-urocanic acid (*trans*-UCA), a molecule which makes up about 0.5% of the total dry weight in the upper layers of human epidermis, to *cis*-urocanic acid (*cis*-UCA) (Noonan, F.P. *et al., J. Invest. Dermatol. 84*:342 abstr. (1985). *cis*-UCA is known to have various immunosuppressive actions *in vivo* in a number of experimental systems (Noonan, F.P. *et al., J. Invest. Dermatol. 90*:92 (1988); Ross, J.A. *et al., J. Invest. Dermatol. 87*:630 (1986); Ross, J.A. *et al., J. Invest. Dermatol. 89*:230 (1987); Ross, J.A. *et al., Viral Immunol. 1*:191 1987/88); Reeve, V.E. *et al., Photochem. Photobiol. 49*:459-464 (1989). *cis*-UCA may act through histamine-like receptors in the skin (Norval, M. *et al., Photodermatol. Photoimmunol. Photomed. 7*:243-248 (1990)). More recently, Kurimoto, I. *et al., J. Immunol. 148*:3072-3078 (1992) showed that the UVB impairment of the induction of contact sensitivity to epicutaneously applied haptens in certain mouse strains depended on the participation of the cytokine, tumor necrosis factor-a (TNFa). The authors suggested that local release of TNFa may inhibit sensitization by trapping epidermal Langerhans cells and preventing them from reaching the draining lymph node where they activate T cells.

Despite the foregoing, it remains unknown whether mast cell degranulators, e.g., *cis*-UCA, can act in the pharmaceutical setting by inhibiting sensitization by drugs applied dermally or transdermally and therefore, whether these materials have utility as immunologically tolerizing and counter-sensitizing agents in man.

3. SUMMARY OF THE INVENTION

Compositions, articles and methods useful for preventing or inhibiting the effects of a skin irritating or skin sensitizing component of a transdermal drug delivery system are disclosed. Agents capable of inducing mast cell degranulation have been found by the present inventors to be useful in inducing immune tolerance, as countersensitizing agents, as well as anti-inflammatory agents.

Thus, present methods include administration before, during or after transdermal drug delivery of a mast cell degranulating agent to effect countersensitization. Alternatively, such agents can be administered prior to, at the onset of, or both prior to and along with, transdermal drug delivery produce a state of immunological tolerance which is sustained and protects the tolerant subject from sensitization upon subsequent exposure to the sensitizing drug or substance. The countersensitizing agent of the present invention is preferably capable of permeating the epidermis and is preferably administered transdermally. Novel methods and compositions containing *cis*-UCA, its analogues or metabolites, and providing anti-inflammatory effect are also disclosed.

Thus the present invention is directed to a method for preventing or inhibiting the skin irritating or skin sensitizing effect of a drug administered to the skin, which method comprises administering before, together with, before and together with, or after the drug, an effective amount of at least one mast cell degranulating agent.

Also provided is a method for preventing or inhibiting the skin irritating or skin sensitizing effect of a component of a transdermal drug delivery system, which method comprises administering before, together with, before and together with, or after the component, an effective amount of at least one mast cell degranulating agent. The skin irritating or sensitizing component may be a transdermally delivered therapeutic drug, a skin permeation enhancing substance, or a combination of the two.

In the above method, the at least one mast cell degranulating agent is preferably administered transdermally, together with the component.

The mast cell mast cell degranulating agent useful in the above methods is preferably selected from the group consisting of: (a) *cis*-urocanic acid, or an analogue or a metabolite thereof; (b) chloroquine; (c) capsaicin; (d) morphine sulfate; (e) a sodium channel ionophore; (f) a calcium channel ionophore; (g) an inhibitor of Na^+/K^+ channel ATPase; (h) quinine; (i) 4-aminopyridine; (j) an anti-human IgE antibody; (k) compound 48/80; (l) substance P; (m) estradiol; (n) somatostatin; (o) clonidine; (p) progesterone; (q) carbachol; and (r) spantide

Preferred *cis*-urocanic acid analogue for use in the above methods include, but are not limited to: (a) a *cis* or trans isomer of 1-furanacrylic acid; (b) a *cis* or trans isomer of 2-pyrrole acrylic acid; (c) a *cis* or trans isomer of 2-thiopheneacrylic acid; and (d) dihydrourocanic acid.

Preferred *cis*-urocanic acid metabolite for use in the above methods include, but are not limited to: (a) histamine; (b) N^1-methylhistamine; (c) N^1-methylhistidine; (d) histidine; (e) imidazolepyruvic acid; (f) N^3-methylhistidine; (g) imidazoleacetic acid; (h) hydantoin 5-propionic acid; and (i) imidazolonepropionic acid.

The above methods are useful for treating or preventing skin irritation or skin sensitization produced by a drug selected from, but not limited to, the following group: (a) an angiotensin converting enzyme inhibitor;

Also provided is a method for inducing immunological tolerance in a subject to a skin sensitizing drug or agent administered to the skin, which method comprises administering to the subject prior to, at the onset of, or both prior to and at the onset of, transdermal delivery of the sensitizing drug or agent, an effective amount of at least one mast cell degranulating agent. The at least one mast cell degranulating agent is preferably selected from the group consisting of *cis*-urocanic acid or an analogue or a metabolite thereof; chloroquine; capsaicin; morphine sulfate; a sodium channel ionophore; a calcium channel ionophore; an inhibitor of Na^+/K^+ channel ATPase; quinine; 4-aminopyridine; an anti-human IgE antibody; compound 48/80; substance P; estradiol; somatostatin; clonidine; progesterone; carbachol; and spantide.

In the above methods, the skin sensitizing drug or agent may be a transdermally delivered therapeutic drug, a skin permeation enhancing substance, an excipient or a combination thereof.

4. DESCRIPTION OF THE FIGURES

FIGURE 1 provides the structural formulae for both the *cis*-UCA (also called the "Z-form" or Z-urocanic acid) and *trans*-UCA (also called the "E-form" or E-urocanic acid). Also shown are the chemical structures of *cis*- and *trans*-UCA as well as a number of analogues of *cis*-UCA.

FIGURE 2 is a graph illustrating Chymase Depletion from Mast Cells by *Cis*-Urocanic Acid.

5. DETAILED DESCRIPTION OF THE INVENTION

The present inventors have discovered that agents capable of inducing a state of immunological tolerance, which renders the immune system specifically unreactive, or hyporeactive, to a particular sensitizing agent for a prolonged period. This class of agents is also capable of inducing mast cell degranulation are useful as countersensitizers when administered before, together with, or after, a transdermally delivered sensitizing drug. Thus one or a few presentations of the agent of the present invention, prior to, along with, or both prior to and along with, the sensitizing agent can render the subject immunologically tolerant to the sensitizing agent.

The present invention is thus directed to a method for preventing or inhibiting the skin irritating or skin sensitizing effect of a component of a dermal or transdermal drug delivery system, the method comprising administering before, together with, or after the component, an effective amount of at least one countersensitizing agent which agent can induce mast cell degranulation.

The skin sensitizing or irritating component of a TDS can be a transdermally delivered therapeutic drug, one or more skin permeation enhancers present in the TDS to enhance uptake of the therapeutic drug, or by a combination of the skin penetration enhancer or enhancers with the therapeutic drug or drugs in the transdermal composition.

Preferably, in the above countersensitization method the countersensitizing, mast cell degranulating agent is administered prior to, or together with, the component.

In the above tolerization method, the agent can be administered prior to, at the onset of, or both prior to and at the onset of, transdermal drug delivery to provide a sustained state of immune tolerance. Such a state of tolerance is characterized by the fact that the initial treatment or treatments with the agent of the present invention, which may be co-delivered with the drug, is sufficient to prevent skin sensitizing effects of any of a number of subsequent transdermal drug deliveries without further need to administer the agent.

Any agent capable of inducing mast cell degranulation can be employed according to the present invention. Suitable agents include, but are not limited to, *cis*-urocanic acid or analogues or metabolites thereof, chloroquine, capsaicin, morphine sulfate, sodium and calcium channel ionophores, inhibitors of Na^+/K^+ channel ATPase (such as tridecylresorcylic acid), quinine, 4-aminopyridine, an anti-human IgE antibody, compound 48/80, substance P, spantide and the like.

Preferably, the countersensitizing and mast cell degranulating agent is able to permeate the epidermis, for example, following administration via transdermal delivery. Accordingly, the preferred agents of the present invention have a molecular weight of 600 daltons or less and have a melting point of about 300 °C or less. Most preferably the agent is *cis*-urocanic acid or an analog or metabolite thereof. Unexpectedly, *cis*-urocanic acid has also been found to provide an anti-inflammatory effect.

5.1. LOCALLY ACTING IMMUNOSUPPRESSIVE AGENTS: COUNTER IRRITANTS AND COUNTER SENSITIZERS

The terms "counter-sensitizer" or "counter-sensitizing agent", as used herein define a class of locally-acting immunosuppressive agents which are characterized by activity of causing degranulation of cutaneous

5.3. SKIN PERMEATION ENHANCERS WHICH IRRITATE OR SENSITIZE TRANSDERMALLY

Skin permeation enhancers, substances that increase the permeability of the skin without severe irritation or damage to its structure (Hadgraft, J., *supra*), are commonly added to a TDS. Permeation enhancers may also induce skin irritation or contact sensitivity in some individuals, either alone or in conjunction with the therapeutic drug with which they are combined.

The present invention is also intended to be used to counteract such unwanted effects of the skin permeation enhancing agents. Non-limiting examples of skin irritating chemical enhancers include ethanol, oleic acid, oleyl alcohol, linoleic acid, propylene glycol, lauramidopropyl betaine, 2-pyrollidone, N-methyl pyrrolidone, N-methyl formamide, dimethylsulfoxide, dimethylformamide, decylmethylsulfoxide, isopropyl alcohol, t-butanol, and sodium lauryl sulfate.

These permeation enhancers can be divided into three basic categories (Barry, B.W., *Int'l. Conf. Controlled Release Bioact. Mater. 13*:136 (1986); Barry, B.W., *J. Controlled Release 6*:85 (1987)):

1. Enhancers which promote permeation through both polar and lipid regions, *e.g.*, 2-pyrollidone, N-methyl pyrollidone and N-methyl formamide;
2. Accelerants which preferentially affect the polar pathway, *e.g.*, propylene glycol plus decylmethyl sulfoxide; and
3. Permeation enhancers which mainly modify the lipid route, e.g., propylene glycol and propylene glycol + oleic acid.

Combinations of permeation enhancers are sometimes necessary to obtain the desired effect.

5.4. APPLICATION OF COUNTER-SENSITIZING AGENTS

One or more of the counter-sensitizing agents of the present invention, as described above, is administered with a therapeutic drug or drug combination. The counter-sensitizing agent may be administered topically. In a preferred embodiment, the counter-sensitizing agent is administered in a transdermal or a controlled-release device. Examples of transdermal devices and delivery systems are disclosed in Bodde, H.E. *et al.*, *Crit. Rev. Ther. Drug Carrier Syst. 6*:87-115 (1989); and in U.S. Patents No. 3,598,122, 3,598,123, 4,286,592, 4,314,557, 4,379,454, 4,559,222, 4,573,995, which references are hereby incorporated by reference.

The precise formulation of the transdermally administered drug and the counter-sensitizing agent of the present invention can be designed to deliver the drug and the counter-sensitizing agent at the desired fluxes and can be in numerous forms, including, without limitation, ointments gels and creams. Aqueous formulations, in particular gels, typically comprise water and about 1-2% (w/w) of a gelling agent such as hydroxyethyl cellulose or hydroxypropyl cellulose. Typical non-aqueous gels comprise silicone fluid or mineral oil. The mineral oil may also have 1-2% (w/w) of a gelling agent such as colloidal silicon dioxide. The suitability of a particular gel composition depends on the compatibility of its constituents with the sensitizing drug (with or without a permeation enhancer) and the counter-sensitizing agent.

In another embodiment, one or more counter-sensitizing agents of the present invention is delivered to the skin prior to the administration of the therapeutic drug or drugs. Such prior administration can be via transdermal application using a device as described above, via direct topical application, intracutaneous injection, and the like.

In yet another embodiment, one or more counter-sensitizing agents is delivered by another non-cutaneous route and method of delivery, either concurrently with, or prior to, the transdermal administration of the therapeutic drug. Examples of such administration include subcutaneous, intravenous, intramuscular, or intraperitoneal routes. Alternatively, or concurrently, administration may be by the oral route.

In all of the above embodiments, the dosage of counter-sensitizing agent administered will be dependent upon the agent, the age, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment.

The methods and compositions within the scope of this invention include all compositions and methods wherein the counter-sensitizing agent is contained in an amount effective to achieve its intended purpose. While individual needs vary, determination of optimal ranges of effective amounts of each component is within the skill of the art.

For transdermal administration, typical effective dosages of the counter-sensitizing agent to prevent sensitization by a sensitizing drug will depend on it permeation through human skin, and is a function of the physical properties of the permeant, including the partition coefficient of the permeant between solvent and skin, molecular weight and melting point. In general, the maximal flux that can be obtained from any permeant occurs from saturated solutions. Equations have been derived that predict accurately the maximal

receptor solution (saline) were collected and the amount of *cis* and *trans* isomers that permeated through the skin determined by standard HPLC methods. One hundred and eight hours post-incubation at 35 °C, the remaining UCA in the donor chamber was collected and the tissue homogenized and extracted with base (0.5N KOH). The tissue extracts were neutralized and aliquots analyzed by HPLC.

The recovery of the *cis*- and *trans*- isomers in the receptor and donor chambers, the amount retained in the tissue, and the total recovery are all shown in Table I, below. The recovery of UCA isomers in this system was quantitative and amounted to over 90% of the applied dose.

Most of the applied UCA acid did not permeate and was recovered in the donor chamber of the diffusion cells. The fraction that permeated accounted for only 1.05% of sample-I, 1.48% of sample-II, and 15.14% of sample-III.

trans-UCA and *cis*-UCA permeated human skin at rates which were functions of dosage form and concentration in the applied dosages. Sample I showed the slowest overall permeation rate of 0.169 $\mu g/cm^2/hr$ for both *trans*- and *cis*-UCA. The combined permeation rate of trans and *cis* UCA from, sample II was 1.08 $\mu g/cm^2/hr$ and that for sample III was 26.74 $\mu g/cm^2/hr$.

TABLE I

SUMMARY OF THE RECOVERY OF UROCANIC ACID ISOMERS IN THE SKIN PERMEATION STUDY						
SAMPLE ID	Amount TESTED (mg/ml)	Z-UCA (%)	RECOVERED IN			Total Recovery %
			Receptor (µg)	Donor (µg)	Tissues (µg)	
I	5.147	67	25	2,318	47	92.85
II	15.624	55	106	7,001	55	91.67
III	36.148	67	2713	13,897	203	93.02

Table II, below shows the fluxes of UCA isomers through human skin. Both *trans*-UCA and *cis*-UCA permeated human skin at comparable fluxes. The overall flux for sample I was 0.17 $\mu g/cm^2/hr$, for sample II 1.08 $\mu g/cm^2/hr$, and 25. 7 $\mu g/cm^2/hr$ for sample III.

The observed fluxes for the *cis*- and *trans*-isomers of UCA were in the range of calculated values (5-10 $\mu g/cm^2/hr$) for flux rates derived from equations developed to predict flux through human skin. For further details see Kydonieus, A. (ed.), *supra*.

TABLE II

SUMMARY OF FLUXES OF UROCANIC ACID ISOMERS THROUGH HUMAN PARTIAL THICKNESS SKIN				
SAMPLE ID	Z-ISOMER ($\mu g/cm^2/hr$)	E-ISOMER ($\mu g/cm^2/hr$)	RATIO (Z/E)	OVERALL FLUX ($\mu g/cm^2/hr$)
I	0.0987	0.0676	1.46	0.1690
II	0.5825	0.4765	1.22	1.0839
III	19.5648	7.2327	2.71	26.7138

EXAMPLE 3

BIOLOGICAL ACTIVITY OF *cis*-UCA: INHIBITION OF CONTACT HYPERSENSITIVITY

The mast cell degranulating agent, *cis*-UCA was tested for its ability to act as a counter-sensitizer.

TEST ANIMALS

BALB/c female mice were maintained in a specific pathogen-free colony. Age-matched mice between 8 and 12 weeks of age were maintained in filter-protected cages and fed mouse chow and water ad libitum. Lighting was controlled to provide 12 hours light-dark cycles.

TABLE III

cis-UROCANIC ACID INHIBITS CONTACT SENSITIVITY: (PRETREATMENT VS PRETREATMENT PLUS CO-ADMINISTRATION)			
TREATMENT	EAR THICKNESS (mm X 10^3)	EAR SWELLING (mm X 10^3)	% SUPPRESSION
NONE (PBS, pH 5.5)			
24 Hours	20.8 ± 2.1	--	--
48 Hours	15.8 ± 3.6	--	--
TNBS ONLY (35μG)			
24 Hours	82.3 ± 7.0	61.5	--
48 Hours	58.3 ± 10.0	42.5	--
cis-UROCANIC ACID (PBS, pH 5.5) (PRE ONLY) plus TNBS (35 μG)			
24 Hours	40.3	19.5	68.3
48 Hours	25.5	9.7	77.2
cis-UROCANIC ACID (PBS, pH 5.5) (PRE + CO-AD) plus TNBS (35 μg)			
24 Hours	49.8	29.0	52.8
48 Hours	34.6	18.8	55.8

Thus, pretreatment with *cis*-UCA before sensitization by TNBS resulted in a 68% and a 77% suppression of the 24 hour and 48 hour ear swelling response, respectively, to challenge with the cross-reacting hapten, TNCB.

In the pretreatment plus co-administration protocol, there was a 53% and a 56% suppression of the 24 hr and 48 hr ear swelling response, respectively, to challenge with TNCB.

D. *cis*-Urocanic Acid Inhibition of Contact Sensitivity is Dose Dependent

A dose-response study of the *cis*-UCA suppression of the contact sensitivity reactions was performed. The results of these experiments are shown in Table IV below.

TABLE IV

cis-UROCANIC ACID INHIBITS CONTACT SENSITIVITY: DOSE RESPONSE EFFECT			
TREATMENT	EAR THICKNESS (mm X 10^3)	EAR SWELLING (mm X 10^3)	% SUPPRESSION
NONE (PBS)	19.3 ± 6.4	--	--
TNBS ONLY (3.5mg)	162.3 ± 21.8	140.2	--
TNBS +			
cis-UCA 200 μg	109.0 ± 8.6	89.7	36.0
cis-UCA 600 μg	71.3 ± 16.2	52.0	63.0
cis-UCA was dissolved in PBS, pH 7.2; ear thickness was measured 24 hours after challenge; N = 5			

TABLE VI

CHLOROQUINE INHIBITION OF CONTACT SENSITIVITY			
TREATMENT	EAR THICKNESS (mm X 10^3)	EAR SWELLING (mm X 10^3)	% SUPPRESSION
NONE (PBS)			
24 Hours	16.8 ± 2.9	--	--
48 Hours	16.8 ± 0.9	--	--
TNBS ONLY (2.45 mG)			
24 Hours	100.6 ± 15.6	83.6	--
48 Hours	160.8 ± 28.1	144	--
TNBS + CHLOROQUINE (200 µG)			
24 Hours	67.4	50.6	39.6
48 Hours	93.8	77.0	46.5
The chloroquine dose, 10% of the LD , was administered intradermally			

Thus, intradermal injection of mice with a sublethal dose of chloroquine (10% of the LD_{50}) suppressed contact sensitivity by 40 - 47%.

EXAMPLE 5

A MAST CELL MEMBRANE STABILIZING AGENT PROMOTES CONTACT SENSITIZATION

In view of the present inventors conception that mast cell degranulating agents act as counter-sensitizers, they further predicted that an agent which stabilizes mast cell membranes would not suppress, and might even enhance contact sensitization.

To test this prediction, sodium cromolyn, which is known to stabilize mast cell membranes (Klein *et al.*, *Proc. Natl. Acad. Sci. USA* 86:8972-8976 (1989)), was tested in the same experiment in which chloroquine was tested above. The results are shown in Table VII, below.

TABLE VII

EFFECT OF CROMOLYN ON CONTACT SENSITIVITY			
TREATMENT	EAR THICKNESS (mm X 10^3)	EAR SWELLING (mm X 10^3)	% SUPPRESSION
NONE (PBS)			
24 Hours	16.8 ± 2.9	--	--
48 Hours	16.8 ± 0.9	--	--
TNBS (2.45 mG)			
24 Hours	100.6 ± 15.6	83.6	--
48 Hours	160.8 ± 28.1	144	--
TNBS plus			
CROMOLYN (20 µG)			
24 Hours	112.4 ± 11.8	95.6	+14.1
48 Hours	185.2 ± 16.1	168.4	+17.0
Cromolyn was injected intradermally.			

B. cis-UCA-CONTAINING PATCH WHICH COUNTERACTS SENSITIZING EFFECT OF THE PERMEATION ENHANCER, OLEIC ACID

Three plastisol formulations containing the drug isosorbide dinitrate (ISDN) and the skin permeation enhancer oleic acid will be prepared esssentially as above for the clemastine patches. The formulations are summarized in Table IX, below.

TABLE IX

ISDN/Oleic Acid Formulation (in parts)			
Component	Formulation		
	1	2	3
PVC Resin	55	51	50
Dioctyl Phthalate	35	31	30
ISDN (Drug)	10	10	10
Oleic Acid (Enhancer)	0	2	2
cis-UCA	0	0	2

When permeation rates are measured through human skin *in vitro*, it is found that the presence of oleic acid significantly enhances the permeation rate of ISDN.

All three patches are placed on the skin of human subjects over a 24 hour period. Patch 1, lacking oleic acid, and Patch 3, containing both oleic acid and *cis*-UCA show minimal irritation and/or sensitization. In contrast, Patch 2, containing oleic acid but no counter-sensitizing agent, induces substantial irritation and/or sensitization.

EXAMPLE 8

PURIFICATION AND CONCENTRATION OF *CIS*-UROCANIC ACID

A. PURIFICATION OF *CIS*-UROCANIC ACID

A solution of photoisomerized *trans*-UA (ratio over 70:30 *cis*- to *trans*-; prepared as described in Example 1) was loaded to an ion exchange chromatographic column packed with Dowex 1-X8 (Biorad) and pre-equilibrated with 0.0125 M ammonium acetate. After washing off unbound materials with 0.050 M solution, *cis*-UA was eluted with a linear gradient of 4L solution consisting of 0.050 M ammonium acetate (2L) and 0.5 M of the same salt. Fractions containing *cis*-UA were identified using a UV-spectrophotometer and confirmed by HPLC. For scale preparation, 5 liters of photoisomerized solution of *trans*-UA was concentrated by lyophilization. The powder was dissolved in 1.25 L of 0.0125 m ammonium acetate buffer, filtered, and applied to 600 ml packed gel. Fractions of 6 ml were collected on a Frac-200 fraction collector (Pharmacia) equipped with a peristaltic pump (P-1) and a 3-way flow diversion valve. Using this system, only fractions of interest were collected.

B. CONCENTRATION OF THE UROCANIC ACID

Solutions of isomers or of *cis*-UA were concentrated in bulk form or in aliquots by lyophilization. For this purpose, the solutions were frozen in suitable containers in a freezing bath at -80 • C for 1.5 hours or in the Unitop chamber (Virtis) at -40 • C for 6 to 8 hours. Following the evacuation, a primary and a secondary lyophilization cycle were used to remove solvent.

The primary cycle consisted of raising the temperature of the sample gradually from -40 • C to 15 • C for a period of 72 hours, and the secondary, and after the sample is dry, from 15 to 35 • C for a period of 48 hours. For a long term storage product, the chamber was filled with nitrogen gas and the vials stoppered using a mechanical stoppering device built in the unitop. The Teflon stoppers on each vial were secured using aluminum caps and a hand-held vial crimper device (Wheaton). Lyophilized samples were stored in the freezer and found to be stable for months under these conditions.

TABLE X

cis-UROCANIC ACID INHIBITS CONTACT SENSITIVITY			
TREATMENT	EAR THICKNESS (mm X 10^3)	EAR SWELLING (mm X 10^3)	% SUPPRESSION
NONE (HPMC GEL)			
24 Hours 72 Hours	244 ± 8 241 ± 4	– –	– –
DNCB ONLY (100 µG)			
24 Hours 72 Hours	332 ± 21 313 ± 32	88.8 72.0	– –
cis-UROCANIC ACID 2 mg (1%) (HPMC GEL) (Petreat) + DNCB (100 µg)			
24 Hours 72 Hours	270 ± 16 288 ± 23	26 48	71 33
cis-UROCANIC ACID 10 mg (5%) (HPMC gel) (Petreat) + DNCB (100 µg)			
24 Hours 72 Hours	261 ± 15 270 ± 15	18 30	80 59

For the 1% CUA gel, 71% and 33% suppression of ear swelling response were observed 24-hours and 48-hours post-challenge, respectively. For a 5% CUA gel, an 80% and 59% suppression of the ear swelling response were obtained at 24 hours and 48 hours, respectively.

b. Co-delivery

In one experiment, a 5% CUA gel was either co-delivered with 1% DNCB or delivered for 24 hours prior to sensitization with 1% DNCB. Sensitization was carried in the gel formulation for 24 hours. The results are seen in Table XI, below.

TABLE XII

COMPARISON OF THE 5 HOURS VERSUS 24 HOURS PRETREATMENT ON IMMUNE SUPPRESSION BY CIS-UROCANIC ACID			
TREATMENT	EAR THICKNESS (mm X 10^3)	EAR SWELLING (mm X 10^3)	% SUPPRESSION
NONE (HPMC GEL) n = 6			
24 Hours 48 Hours	237 ± 8 236 ± 15	-- --	-- --
DNCB only (100 µg) for 5 hours Pretreat n = 8			
24 Hours 48 Hours	320 ± 34 299 ± 30	83 63	-- --
DNCB only (100 µg) for 24 hours Pretreat n = 8			
24 Hours 48 Hours	353 ± 30 316 ± 33	116 80	-- --
5 Hours Pretreat (UCA 15 mg) + DNCB (100 µg) n = 8			
24 Hours 48 Hours	285 ± 27 284 ± 20	42 42	46 27
24 Hours Pretreat (UCA 15 mg) + DNCB (100 µg) n = 8			
24 Hours 48 Hours	288 ± 25 276 ± 14	47 36	59 54

A significant inhibition of 46% and 27% at 24-hours and 48-hours post-challenge, respectively was observed for the 5-hour pretreat regime. This compares favorably with the results obtained using the standard 24-hour pretreatment regime, which gave 59% and 54% inhibition for the 24-hour and 48-hour post-challenge, respectively.

2. *Cis*-Urocanic acid induces a state of immune tolerance

Balb/c mice (N=8) were pretreated for 24 hours with 1% CUA in HPMC gel or 5% CUA in HPMC gel on the shaved abdominal skin (site 1), and challenged on the right ear five days later. The CUA-treated mice were immunosuppressed as shown in Table X. The same mice were re-sensitized with DNCB on a demarcated area of their shaved backs and re-challenged five days later on their left ears, and the ear thickness measured 24 hours post-challenge.

The results of this experiment are presented in Table XIII, below.

Claims

1. An article useful for preventing or inhibiting the skin irritating or skin sensitizing effect of a component of a dermal or transdermal drug delivery system, said article comprising:
   (a) a dermal or transdermal delivery system comprising a therapeutic drug of interest; and
   (b) an effective amount of at least one mast cell degranulating agent.

2. An article according to claim 1, wherein said mast cell degranulating agent is included in the transdermal delivery system (a).

3. An article according to claim 1 or claim 2 wherein the mast cell degranulating agent is selected from the group consisting of cis-urocanic acid or an analogue or a metabolite thereof; chloroquine; capsaicin; morphine sulfate; a sodium channel ionophore; a calcium channel ionophore; an inhibitor of Na^+/K^+0 channel ATPase; quinine; 4-aminopyridine; an anti-human IgE antibody; compound 48/80; substance P; estradiol; somatostatin; clonidine; progesterone; carbachol; and spantide.

4. An article according to any preceding claim wherein said at least one mast cell degranulating agent is cis-urocanic acid, or an analogue or a metabolite thereof.

5. An article according to any preceding claim wherein said drug of interest is selected from the group consisting of an angiotensin converting enzyme inhibitor; a beta adrenergic receptor blocker; an anti-hypertensive drug other than an angiotensin converting enzyme inhibitor or a beta adrenergic receptor blocker; an anti-histamine; an anti-asthmatic drug; a non-steroidal anti-inflammatory drug; a central nervous system active drug; a weight control drug; an anticoagulant; a potassium control drug; and a decongestant.

6. An article according to claim 1, wherein the transdermal delivery system (a) further comprises a skin permeation enhancing substance.

7. An article according to claim 6, wherein said skin permeation enhancing substance is selected from the group consisting of ethanol, oleic acid, oleyl alcohol, linoleic acid, propylene glycol, lauramidopropyl betaine, dimethylsulfoxide, dimethylformamide, decylemthylsulfoxide, N-methylpyrrolidone, isopropyl alcohol, t-butanol, and sodium lauryl sulfate.

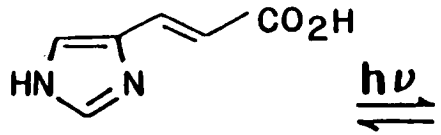
8. A pharmaceutical composition useful for preventing or inhibiting the skin irritating or skin sensitizing effect of a component of a dermal or transdermal drug delivery system, said composition comprising:
   an effective amount of at least one mast cell degranulating agent and an acceptable pharmaceutical carrier.

9. A pharmaceutical composition according to claim 8, wherein the mast cell degranulating agent is cis-urocanic acid, or an analogue or a metabolite thereof.

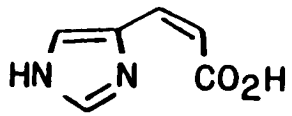
10. A pharmaceutical composition according to claim 8 or 9, wherein the cis-urocanic acid analogue is selected from the group consisting of a cis or trans isomer of 1-furanacrylic acid, a cis or trans isomer of 2-pyrrole acrylic acid, a cis or trans isomer of 2-thiopheneacrylic acid, and dihydrourocanic acid.

11. A pharmaceutical composition according to any preceding claim wherein the cis-urocanic acid metabolite is selected from group consisting of histamine, N^1-methylhistamine, N^1-methylhistidine, histidine, imidazolepyruvic acid, N^3-methylhistidine, imidazoleacetic acid, hydantoin 5-propionic acid, and imidazolonepropionic acid.

12. A pharmaceutical composition according to any prededing claim, wherein, the counter-sensitizing agent is in a form for dermal or transdermal administration.

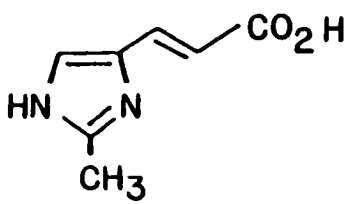


transurocanic acid(1)

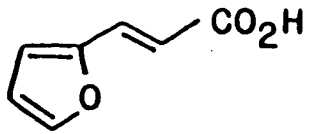


cisurocanic acid(1)

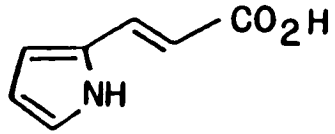
FIG. 1



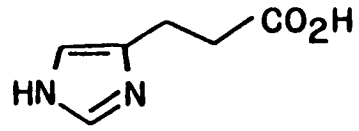
2-methylurocanic acid(2)



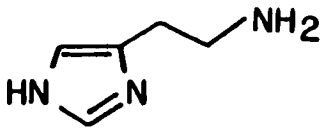
2-furanacrylic acid(3)



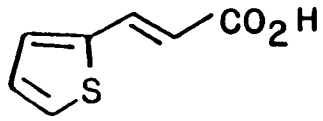
2-pyrroleacrylic acid(4)



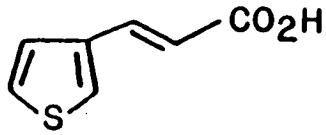
dihydrourocanic acid(5)



histamine(6)



2-thiopheneacrylic acid (7)



3-thiopheneacrylic acid(8)

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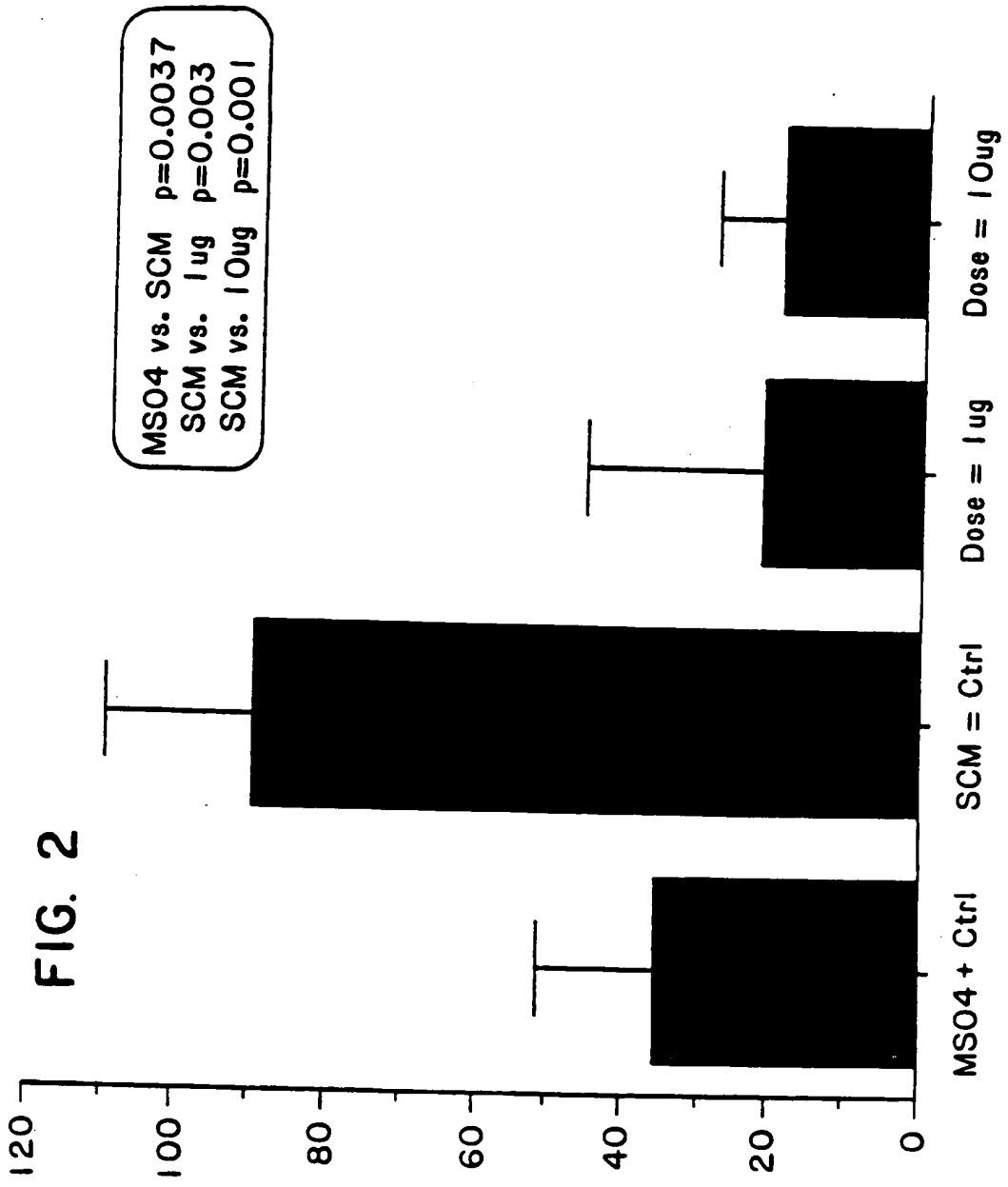
FIG. 2


MSO4 vs. SCM p=0.0037
SCM vs. 1ug p=0.003
SCM vs. 10ug p=0.001
120
100
80
60
40
20
0
MSO4 + Ctrl
SCM = Ctrl
Dose = 1ug
Dose = 10ug

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 94 20 0484

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.5)
X	EP-A-0 467 116 (BODE CHEMIE GMBH & CO) 22 January 1992 * page 2, line 1 - line 9 *	1	A61K31/47 A61K31/415 //(A61K31/47, 31:415,31:40, 31:34)

TECHNICAL FIELDS SEARCHED (Int.Cl.5)

A61K

INCOMPLETE SEARCH

The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

see sheet C

Place of search	Date of completion of the search	Examiner
THE HAGUE	1 June 1994	Leherte, C

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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EP 94 20 0484

-C-

INCOMPLETE SEARCH

Claims searched incompletely: 1-12

In view of the large number of compounds which are defined by the wording of the claims, the search has been performed on the general idea and compounds mentioned in the examples of the description (EP art. 84, Guidelines, Part B, chapt. II. 7 last sentence and chapt. III.3.7.).